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THE QUALITY OF SEXED SEMEN ON FILIAL ONGOLE BULL USING PERCOLL DENSITY GRADIENT CENTRIFUGATION METHOD

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Abstract-Artificial Insemination technology can be increased in value by using sexed semen program that produce sex of calf as expected. The purpose of this study to determine the quality of sexed semen using percoll density gradien centrifugation method in Filial Ongole Cattle. Semen was collected using Artificial Vagina (AV) from Filial Ongole Cattle, evaluated and then separated using percoll density gradien centrifugation method (PDGC). Only fresh semen with a minimum of 70% individual motile sperm and 2+ mass of motility used in this study. Andromed as a based extender was diluted using aquabidest with 1:4 ratios. The obtained data were analyze with analysis of variance (ANOVA) and continued by Duncan test if there was significant or very significant different. The result showed that the semen quality of non-sexed sperms, upper (Y sperms) and under (X sperms) fraction using SGDP had very significant different (P<0,01) on motility (64,25±3,94%; 53±7,93%; 48,55±8,28% respectively), viability (95,07±0,99%; 88,64±0,51%; 85,15±0,84% respectively), abnormality (0,93±0,28%; 1,98±0,27%; 4,42±0,36% respectively). The study concludes that the sexed semen using percoll density gradien centrifugation method can be used as an alternative separation of X and Y sperms.

INTRODUCTION

In order to improve the genetic quality and productivity of beef cattle, while this has been done is the application of Artificial Insemination (AI). The AI technology can be increased in value by using a program that produced calves have sex-matched expectations with the sexed sperms, because it supports the breeding program in the selection of breeds. Besides the advantage sexed sperms capable of supporting efficiency in beef cattle breeding, because it can obtain a calf with a specific sex in accordance with the development of these farms. The pregnancy rate results AI with

frozen semen without sexing was generating 59,25%, while using percoll density gradient centrifugation sexed semen produce 51,85% (Susilawati et al., 2015).

Percoll density gradient centrifugation was used as a way to separate the X and Y sperms. Percoll is a medium that can be made with different density, it does not penetrate the cell membrane and has a low viscosity (Susilawati, 2014). The results showed that the method of separation by percoll density gradient centrifugation have the opportunity to develop. This is evidenced by the ability of the sperms fertilizing with ovum, showing the sex of calves in line with expectations (Susilawati, 2005; Wahyudi et al., 2014).

Centrifugation and the freezing process can lead to stress on sperm cells that often produce membrane damage and decrease in motility and viability of sperms (Nishizono et al., 2004; Gadea et al., 2005). To be able to maintain the quality of semen in order to remain well after centrifugation, during cooling or freezing it is necessary diluent and freezing the right, so that sperms do not experiencing cold shock. In fresh semen contained 69,16 ± 8,85% sperms are intact, it indicates that the sperms in a fresh state still a lot intact acrosome. While in the frozen semen increased the number of sperm in ½ phosphorescent head end, it indicates that the freezing process triggers the acrosome reaction or not intact acrosome, although only a few are undergoing the acrosome reaction (Diliyana, et al., 2014). Therefore important to know the quality of sexed semen process results in Filial Ongole bull.

MATERIALS AND RESEARCH METHODS

1. Research material

The semen used was fresh semen originating from PO cattle that reared in the Beef Cattle research Area Grati Pasuruan with mass motility criteria ≥ 2+, individual motility ≥70%, 3-5 years old and weighing 450-650 kg. The feed that given is *yellow feed.* Shelter frequency is twice per week for each individual.

2. Research methods

This study was a laboratory experimental study that freezing sexed semen using percoll density gradient centrifugation. Separation method of X and Y sperms using medium density percoll 10 arranged from highest to lowest density (65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%) and centrifugation 2250 rpm for 5 minutes (Susilawati et al., 2000). The extender that used was AndroMed®, aquabidest added with a ratio between Andromed and aquabidest was 1 : 4.

The research design used randomized group design. The parameters observed in this study, such as motility, viability and abnormality. Individual motility of sperms expressed in percentage of sperms actively moving forward in a field of view of 100-200 cells were observed using a light microscope at 400 times magnification slide closed by cover glass (Asadpur, 2012). Viability of sperms expressed in percentage of live sperms count of 200 observation of sperms by using a light microscope magnification of 400 times. A drop of semen is placed at the threshold of object glass, then a drop of eosin negrosin solution placed close together and homogenized. Another object glass used to shift the solution that has been mixed with a slope angle of 30^{o}C and dried over a bunsen lamps, and then observed under a microscope. The sperms that red is dead, while the white ones are living (Susilawati, 2011). Abnormalities expressed in percentage of abnormal sperms count of 200 sperms observations by using a light microscope magnification of 400 times (Asadpur, 2012). Data were analyzed using analysis of variance. If there is significant or very significant then do further testing using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

1. Fresh Semen Quality

Terms of semen quality may be used in this research was to have motility >70%, cattle were used in this study has a standard percentage motility of 70%, and no more than 70% as shown in Table 1.

Table 1. The PO Cattle Semen Quality when the Study of Sexed Semen

Parameter	Value
Volume (ml)	4,4 ± 2,18
Motility (%)	70 ± 0
Viability (%)	95,12 ± 0,98
Abnormality (%)	1 ± 0
Concentration	1.758 ± 137,66
Total motile sperm	536.130 ± 255,812

Percentage motility standard semen used in the study was 70%, this corresponds to Ax et al. (2008) found a decent standard of sperm in the further process was 70%, this is because the percentage motility of sperms that have below 60% the results will not meet the standards in the process before freezing up in the freezing process. The percentage of fresh semen abnormalities 1 ± 0 % showed that fresh semen was used for further processing feasible because according Susilawati (2011) and Ax et al. (2008) sperm abnormalities may not exceed 20%. The percentage of sperm viability fresh semen was 95,12 ± 0,98 % which was still within in the normal range and was high such as research (Susilawati, et al., 2014) shows the percentage of 80% and the percentage motility abnormalities 6,52%. Semen concentration values obtained in this study is 1.758±137,66 x 10^6/ml which shows that the value of the concentration of relatively normal because, according to Garner and Hafez (2008) sperm concentration of cattle is 800-2000 x 10^6/ml. Examination of the concentration needs to be done because the sperm concentration can be used to predict the fertility of cattle (Ax et al., 2008). The quality of fresh semen used in this study was the semen that has good quality, it was intended that the sperms were able to survive during the separation process with percoll density gradient centrifugation methods.

2. Sexed Semen Quality

The percentage motility and viability of semen after sexing decreased in all treatments shown in Table 2.

Table 2. Percentage of The Sperms Motility, Viability and Abnormality of Semen after Sexing

Treatment	Motility	Viability	Abnormality
Non Sexing	64,25 ± 3,94^b	95,07 ± 0,99^b	0,93 ± 0,28^a
Sexing SGDP (X)	53 ± 7,93^a	88,64 ± 0,51^a	1,98 ± 0,27^b
Sexing SGDP (Y)	48,55 ± 8,28^a	85,15 ± 0,84^a	4,42 ± 0,36^c

Table 2. show that the sexed motility and viability results show a decline. Seen that sexed motility and viability results show the percentage of X sperms higher than Y sperms. But non sexed semen still shows the percentage motility better (P<0,01) compared sexed semen.

Kusumawati, et al. (2016) found in the bottom layer contains 72,0±2,26% X sperms, while the upper layer contains 77,5±1,26% Y sperms. Y sperms head are usually smaller, lighter and shorter than the X sperms, so that the Y sperms is faster and more mobile, and X sperms contains more DNA than Y sperms that produce bulls. Thus, if a centrifuge, the X sperms are more likely to form a precipitate compared with Y sperms. This was caused by differences in age and size of the Y sperms are smaller than the X sperms, so that the movement of the Y

sperms is faster and has a higher penetration power to enter into a liquid. X sperms chromatin containing more in his head, resulting in head size of X sperms become larger (Garner and Hafez, 2008; Hafez and Hafez, 2008).

Reduced motility occur because of various treatments, among others, the process of separation, washing and cooling that causes sperm requires a lot of energy to maintain physiological conditions. In addition, due to the circular motion that occurs in sperms due to centrifugal force so that the sperms damaged. This is in accordance opinions Susilawati (2003) and Susilawati et al. (2014) also states that centrifugation can lead to damage cell membranes of sperms. The function of the membrane itself is as protectors of cells, so that when the membrane is damaged, it can cause damage to the organelles found in cells such as mitochondria and lysosomes. Mitochondria are the venue for cell respiration to produce energy, so that in case of damage can interfere with the metabolic processes that will affect the movement of sperms.

X sperms viability after sexing process was higher than Y sperms with each value of more than 80%. It is still said to be normal as the research results Susilawati, et al. (2014) that the results of sexing by 5 minutes centrifugation showed that the sperm viability in the upper layer (Y) of 75,27% and the bottom layer (X) as 79,68%. The decline in the percentage of sperm after separation can be caused by the separation of sperm from seminal plasma, as well as sperm medium, seminal plasma also serves as a source of energy. If the energy supply needed sperm less it will disturb the viability of sperm. According Susilawati (2003) seminal plasma that typically contain citric acid, ergotionine, fructose, phosphorylcholine glysery and sorbitol. Fructose in spermatozoa metabolic processes are useful as a source of energy.

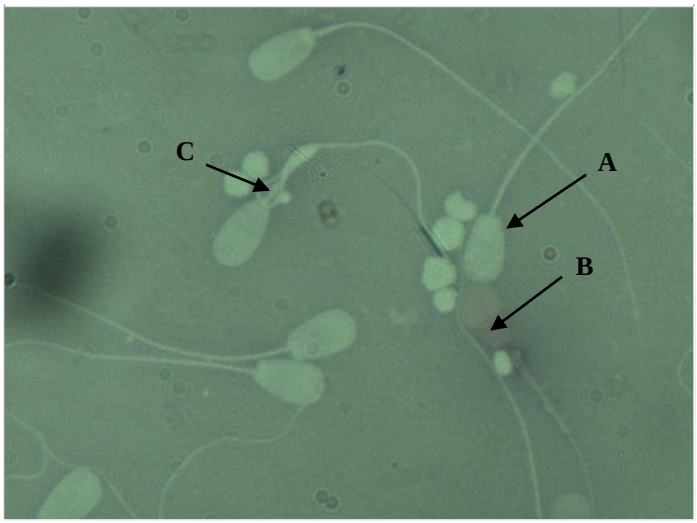

Fig. 1. Live spermatozoa (A), death (B) and abnormal (C)

During centrifugation, the centrifugal force causes the sperm to collide with the tube wall and medium, as a result of the collision damaged sperm, at high speed centrifugation of sperm not only damage alone but it is possible that sperm destroyed. Sperm vitality depends on the integrity of the membrane. According Susilawati (2000) that the separation of sperm with centrifugation method resulted in damage to the structure of the membrane of sperm. The membrane damage occurs as a result of Reactive Oxygen Species (ROS). ROS are free radicals that play an important role in physiological processes such as sperm capacitation, hyperaktivasi and *sperm-oocytes fusion* (Aitken et al., 2012; Ball, 2008; Bansal and Bilaspuri 2011) Sperm membrane damage resulted in disruption of sperm intracellular metabolic processes, in the

presence of metabolic disorders, the sperm will be weakened and could even cause death.

The impact of the sexing process, cooling and freezing will increase sperm abnormalities. Most of the increase was the result of a process abnormalities sexing namely centrifugation, refrigeration and freezing is cold shock.

Table 2. show that abnormalities sexing results show improvement. The abnormality of X Sperm was lower (P<0,01) compared Y Sperm. But without sexing semen still showed a lower percentage of abnormalities (P<0,01) compared sexing semen. The sperm abnormality after sexing process in the normal range because not more than 20%. This is supported with the opinion Ax et al. (2008) if the number of sperm abnormality was very high it will reduce the level of fertility of the sperm.

The abnormalities was increased occur for a variety of treatments ranging from the process of separation, washing, refrigeration and freezing which causes sperm requires a lot of energy to maintain physiological conditions. In addition, due to the circular motion that occurs in sperm due to centrifugal force so that the sperm damaged. This is in accordance opinions that centrifugation cause damage of the sperm membrane so that sperm quality decreases with an increase in the number of membrane damage (Susilawati et al., 2014).

CONCLUSION

Based on these results it can be concluded that the quality of sperm sexing of Filial Ongole Bull using percoll density gradient centrifugation method shows the results still good for further process, namely for liquid semen or frozen semen.

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REFERENCES

Aitken R. J., Jones K. T. and Robertson S. A.. 2012. Reactive Oxygen Species and Review Sperm Function-In Stickness and In Health, J. Androl 22 (6): 1096-1106.

Asadpur R. 2012. Relationship between Mineral Composition of Seminal Plasma and Semen Quality in Various Ram Breeds. Acta Scientiae Veterinariae, 40 (2): 1-8.

Ax, R.L., M.R. Dally, B.A. Didion, R.W. Lenz, C.C. Love. D.D. Varner, B. Hafez and M.E. Bellin. 2008. Artifical Ainsemination. In: Reproduction In Farm Animals. E.S.E Hafez and B. Hafez. (Edit). 7^{th} ed. Blackwell Publishing. Australia: 365-375.

Ball B.A. 2008. Oxidative Stress, Osmotic Stress and apoptosis: Impact on Sperm Function and Preservation in The Horse. Animal Reproduction Science 107: 257-267.

Bansal A.K. and Bilaspuri G. S. 2011. Impact of Oxidative Stress and Antioxidants on Semen Function: Review Article. SAGE - Hindawi Access to Research. Veterinary Medicine International: 1-7.

Diliyana Y. F., Susilawati T and Rahayu S. 2014. The Membrane Integrity of The Spermatozoa Sequenced Percoll Density Gradient Centrifugation Air of Diluents Andromed and CEP-2 Were Added Yolk. Journal Veteriner. 15 (1): 23-30.

Gadea, J., Vasquez, F.G., Matas C., Gardon J. C., Canovas, S and Gumbao D. 2005. Cooling and Freezing of Boar Spermatozoa: Supplementation of the Freezing Media With Reduced Glutatione Preserves Sperm Function. Journal of Andrology. 26 (3).

Garner, D.L. and E.S.E. Hafez. 2008. Spermatozoa and Seminal Plasma. In: Reproduction In Farm Animals. E.S.E Hafez and B. Hafez. (Edit). 7^{th}

ed. Blackwell Publishing. Australia: 96-109.

Hafez, ESE and B. Hafez. 2008. X and Y Chromosome-Bearing Spermatozoa in Animal Reproduction in Farm Animal ed by ESE Hafez and B Hafez 7th edition Blackwell Publishing: 390-394.

Kusumawati, E. D., N. Isnaini, A. P. A. Yekti, N. Sholikah, M. Luthfi, L. Affandhy, D. Pamungkas, Kuswati, A. Ridhowi, H. Sudarwati, T. Susilawati. 2016. The Effect of Sexed Semen Methods toward Motility and Ratio of X and Y Sperm Filial Ongole Cattle. The 17th Asian-Australasian Association of Animal Production Societies Animal Science Congress. Proceedings. 22-25 Agustus 2016. Fukuoka Japan.342-344.

Nishizono, H., Shioda M., Takeo T., Irie, T., Nakagata N. 2004. Decrease of Fertilizing Ability of Mouse Spermatozoa after Freezing and Thawing is related to Cellular Injury. Biology of Reproduction. 71: 973-978.

Susilawati T., Hardjopranjoto S, Sumitro SB, Hinting A, 2000. Changes of Membrane Function Cattle Spermatozoa Percoll Density Gradient Centrifugation Results on Gender Selection Process. J. Ternak Tropika. Vol.1

Susilawati, T. 2003. Changes of Membrane Function Cattle Spermatozoa Percoll Density Gradient Centrifugation. Journal Widya Agrika.11 (1).

Susilawati T. 2005. Gestation Success Rate and Accuracy Gender Results of Artificial Insemination Using Frozen Semen Sexing the Filial Ongole Cattle. Journal Animal. Production ,7 (3) : 161 -167

Susilawati, T. 2011. Spermatology.UB press. Malang ISBN 978-602-8960-04-5.

Susilawati, T. 2014. Spermatozoa Sexing (Research Result Laboratory and Application on Cows and Goats). Malang: Universitas Brawijaya Press.

Susilawati, T., S. Rahayu, S. Udrayana, H. Sudarwati, E. Nugroho. 2014. Effect of Different Centrifugation Duration on Simmental Bull Sperm Quality and Membrane Status after Sexing, Cooling and Freezing Processes. American-Eurasian Journal of Sustainable Agriculture. 8(7): 28-34.

Susilawati T., Sri R., Setyabudi U., Herni S., and Eko N. 2014. Effect of Different Centrifugation on Simmental Bull Sperm Quality and Membrane Status after Sexing, Cooling and Freezing Processes. American-Eurasian Journal of Sustainable Agriculture. 8(7):28-34.

Susilawati T., Lieyo W., Nurul I. Aulia P.A.Y. 2015. Reproductive Performance of Ongole Crossbreed Cattle Using Artificial Insemination Sexed Semen With Different Methods. In: Rima A.E.W., Prisilia P.S., Miftahush S.H., Septi M., Aditya A., Riyan N.A., Pradiptya A.H., Satyaguna R., Zefanya A.G., Bagas P., editors. Integrated Approach in Developing Sustainable Tropical Animal Production. Proceeding The 6th International Seminar on Tropical Animal Production. Yogyakarta, 20-22 October. 2015. Yogyakarta (Indonesia): Faculty of Animal Science, Gadjah Mada University, Indonesia. p. 521-525.

Wahyudi, L., T. Susilawati., N. Isnaini. 2014. Reproduction Display of The Result of Artificial Insemination using Frozen Semen Sexing Results in Filial Ongole Cattle on Farms. Journal of Ternak Tropika. 15 (1): 80-88.